REMARKS

This Reply is responsive to the Office Action dated January 30, 2002. Entry of the foregoing and reconsideration on the merits pursuant to 37 CFR 1.112 is respectfully requested.

Support for the Amendments of the Specification:

The specification has been amended as set forth above. In accordance with the new rules for amending applications set forth in 37 CFR 1.121, which took effect on March 1, 2001, marked-up versions of the amended paragraphs showing the changes made are attached hereto as an appendix.

Paragraphs beginning on pages 14, 23, 28, 32, 32, 35, and 37 are amended to correct typographic errors.

The paragraph beginning on page 45 that describes a study of cross-species nuclear transfer by Wolfe et al. (*Theriogenology*, 33:350 (1990)) is amended to avoid incorrectly implying that Wolfe et al. obtained a cross-species blastocyst only when a bovine nucleus was transferred into a buffalo nucleus. Wolfe et al. reported that in addition to the blastocyst obtained from the bovine/ buffalo NT unit, four of the NT units that they obtained by transferring bovine nuclei into goat oocytes cleaved to produce multicellular cross-species embryos, and that one of these progressed to form a "morphologically normal blastocyst" (see the text under the table).

The paragraph bridging pages 45-46 that discloses four oligonucleotide sequences used as PCR primers is amended by inserting a SEQ ID NO after each sequence, in compliance with 37 C.F.R. § 1.821(d).

No new matter was added by way of any of the above amendments.

Amendment of the Claims:

Original claims 1-50 are canceled and new claims 51-131 are submitted. The new claims are limited to subject matter encompassed by 1-25 and 31-50 that were examined and addressed in the Office Action dated January 30, 2002.

Support for the New Claims in the Specification and in the Original Claims:

The new claims are limited to methods using differentiated mammalian donor cells and mammalian recipient oocytes; and to cells produced by the disclosed methods which are not themselves embryos. Claims specifying pluripotent or totipotent embryonic stem cells are withdrawn, and will be re-submitted in a different application. The specification teaches that the embryonic cells produced according to the claimed methods are useful, for example, in studying cell differentiation (e.g., see page 10, lines 1-2).

New independent claim 51, and dependent claims 52-75, 79-85, and 121-124 are directed to a method comprising transferring the genome of a differentiated donor cell of one mammalian species into a recipient oocyte of a different mammalian species to produce an activated nuclear transfer unit capable of dividing to form a multicellular structure, e.g., a blastocyst, and isolating and culturing embryonic cells from the resulting structure. Support for these claims is found, for example, at page 16, lines 1-14, and in original claims 1-14 and 17-23.

New independent claim 86, and dependent claims 76-78, 86-120, and 125-131 are directed to a similar method wherein the genome of the differentiated donor cell that is transferred into the recipient oocyte is genetically altered, e.g., by addition, modification, substitution, or deletion of one or more genes that encode an enzyme, a growth factor, or a

cytokine. Support for these claims is found, for example, at page 25, lines 13-26, and in original claims 31-35.

Support for the use of a differentiated somatic donor cell from an adult human as recited in new claims 52, 55, 57, and 58 is found, for example, at page 39, line 17, to page 40, line 25, and at page 42, lines 14-18.

Support for the method wherein the differentiated donor cell is a germ cell or a somatic cell, as recited in claims 56 and 57 is found, for example, at page 17, line 9.

Support for the method wherein the differentiated donor cell is a cell of the type recited in claims 58-60 is found, for example, in the paragraph bridging pages 16-17.

Support for the method wherein the differentiated donor cell is a human cell, e.g., a human epithelial cell or a human keratinocyte, as recited in claims 61-62, is found, for example, at page 17, lines 16-20, and in Example 1.

Support for the method wherein the differentiated donor cell is from an ungulate as recited in claim 63 is found, for example, at page 16, lines 16-22.

Support for the method wherein the oocyte is from one of the mammals recited in claims 64-68 is found, for example, in the paragraph bridging pages 18-19.

Support for the method wherein the differentiated donor cell is a human cell and the oocyte is a bovine oocyte as recited in claim 69 is found, for example, in Example 1.

Support for the method wherein the activated nuclear transfer unit is cultured on a feeder layer of fibroblast cells to produce a multicellular structure as recited in claim 70 is found, for example, at page 24, lines 20-22.

Support for the method wherein an embryonic cell is isolated from the multicellular structure as recited in claim 71 is found, for example, at lines 23-26 of page 40 (Example 1).

Support for the method wherein an embryonic cell is isolated from a multicellular structure of about 2 to 400 cells as recited in claim 72 is found, for example, at lines 20-24 of page 24.

Support for the method in which the activated nuclear transfer unit is cultured to produce a blastocyst from which embryonic cells are isolated, and cell lines are produced from the isolated embryonic cells, as recited in claims 73-75, is found, for example, in Example 1, which discloses producing such a line of cells having a human genome and bovine mitochondria.The new claims do not contain new matter.

Regarding Rejection of the Claims for Provisional Obviousness-type Double Patenting:

Claims 1-25 and 31-50 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims of co-pending Application No. 09/260,468. Because this is a provisional rejection and the claims may be amended through the course of prosecution, Applicants respectfully request that this rejection be held in abeyance until allowance is negotiated. At that time, if the claims in the instant application and those of the related application are still deemed to be obvious over each other, Applicants will consider submitting a terminal disclaimer.

The examiner's attention is directed to co-pending U.S. Application No. 09/809,018, filed March 16, 2001, and to co-pending U.S. Application No. 09/874,040, filed June 6, 2001, both of which are also related to the present application. In the event that the claims of these related applications also raise double-patenting issues, Applicants respectfully request that rejection on such grounds similarly be held in abeyance until allowance is negotiated.

Regarding Rejection of the Claims Under 35 U.S.C. §101:

Claims 18-23, directed to embryonic or stem-like cells, were rejected under 35 U.S.C. §101 as being directed to non-statutory subject matter, because the claims may be construed to read on a cell that is a human embryo.

New claims 79-85, 112-124, and 128-131 correspond to canceled claims 18-23 in being drawn to isolated embryonic cells having genomic DNA of one mammalian species and mitochondria of a different mammalian species; however, claims 79-85 and 112-120 expressly recite that the claimed cell is not an itself an embryo, and the cells recited in claims 121-124 and 128-131 are isolated cells propagated as a cell line. The claimed cells do not encompass a human zygote, and do not cleave and proceed to develop into an embryonic human being. Accordingly, Applicants submit that the isolated cells recited in the new claims are statutory subject matter under 35 U.S.C. §101, and respectfully request withdrawal of the rejection.

Regarding Rejection of the Claims Under 35 U.S.C. §112, First Paragraph:

Claims 1-17 and 32-45 were rejected under 35 U.S.C. §112, first paragraph, because the specification discloses generation of a fully developed mammal (gaur) from an embryo generated by cross-species nuclear transfer according to the methods of the present invention, and it discloses isolation of a line of embryonic cells produced by culturing embryonic cells derived from a blastocyst generated by cross-species nuclear transfer according to the methods of the present invention, but the application does not demonstrate the pluripotency or totipotency of the such an embryonic cell line by showing that they differentiate into other multilineage cell-types.

The Applicants respectfully traverse the rejection of the claims as non-enabled under 35 U.S.C. §112, 1st Paragraph, and contend that the specification enables one skilled in the art to make and use the claimed invention without undue experimentation

The present claims are directed to a method for generating a nuclear transfer unit by cross-species nuclear transfer, using a differentiated mammalian cell as the nuclear donor cell. Prior to the present invention, it had not been shown that a blastocyst could be produced cross-species nuclear transfer, using a differentiated mammalian cell as the nuclear donor cell, and that lines of embryonic cells could be produced by culturing embryonic cells derived from such a blastocyst.

Wolfe et al. disclose generation of blastocysts from cross-species nuclear transfer units generated by transferring goat and bison cells into enucleated bovine oocytes. However, Wolfe et al. used embryonic goat and bison cells as the donor cells. Those skilled in the art recognize that the chromatin of embryonic donor cells is already in an embryonic configuration, and does not require significant reprogramming by the oocyte cytoplasm to be capable of directing embryogenesis. In contrast, the chromosomes of differentiated donor cells are in a non-embryonic state. One skilled in the art knows that in order for the DNA of a differentiated donor cell to successfully direct embryogenesis, the chromatin of the donor cell must be reprogrammed by the oocyte cytoplasm, the factors in the oocyte cytoplasm that activate expression of genomic genes required for embryogenesis must be compatible with the chromatin proteins and DNA sequences of the donor cell chromatin, proteins encoded by the donor chromatin must be metabolically compatible with proteins and organelles such as mitochondria of the oocyte; and the reprogramming and activation of the donor cell chromatin must occur with timing that is conducive to successful blastogenesis.

Because humans and bovines are so far removed from each other evolutionarily, one skilled in the art would reasonably regard the Applicants' demonstration that a blastocyst can

be successfully generated from a nuclear transfer unit produced by transferring a human differentiated cell into a bovine oocyte, and their successful isolation of a line of embryonic cells having the appearance of ES cells from such a blastocyst, as pretty strong evidence that other cross-species combinations using species that are more evolutionarily related would also yield similar results, as disclosed in the present application. In fact, in addition to the disclosed example, whereby a blastocyst is successfully generated following transfer of skin cell nuclei of a gaur (a species of wild cattle) into enucleated bovine oocytes, results described in scientific articles published subsequent to the priority date of the present application have upheld the teaching of the present application that a nuclear transfer unit capable of generating a blastocyst can be successfully produced transferring differentiated cells of many different mammalian species into an oocyte of a different mammalian species.

For example, Kitiyanant et al. describe the successful generation of blastocysts following transfer of nuclei of fetal buffalo fibroblasts into enucleated bovine oocytes (Cloning Stem Cells, 3(3):97-104, 2001, abstract attached). Trivedi describes work by Loi et al. (published in Nature Biotechnology, 19:962-964, 2001), who successfully generated blastocysts following transfer of granulosa cell nuclei of the European mouflon, a species of sheep, into enucleated oocytes of domestic sheep, a different species (National Geographic Today, October 29, 2001). Dayuan et al. describe the successful generation of blastocysts following transfer of somatic cell nuclei of the giant panda into enucleated rabbit oocytes (Science in China, Series C, 42(4):346-353, 1999, copy attached). Dominko et al. show that transfer of somatic, differentiated cell nuclei of sheep, pigs, and monkeys into bovine oocytes leads to embryogenesis resulting in formation of a "blastocyst-like structure with distinct blastocyst morphology," including an inner cell mass, trophectoderm, and blastocoel cavity (Biology of Reproduction 60:1496-1502, 1999; see page 1500). That one skilled in the art would regard the claimed invention as enabled is further indicated by the statement by

Dominko et al. that embryonic cell lines grown from cross-species embryos such as those of the present invention are expected to be useful for evaluating "long- and short-term effects of mixing of nuclear and cytoplasmic components of various species" (Dominko et al., page 1501). In view of the above, Applicants respectfully request eeconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Regarding Rejection of the Claims Under 35 U.S.C. §112, Second Paragraph:

Claims 1-25 and 31-50 were rejected under 35 U.S.C. §112, second paragraph, because the meanings of the words "embryonic or stem-like cells" and "desired" were considered to be indefinite; because there was no antecedent basis for limitations in claims 31 and 32, and because the meaning of a phrase in claim 46 reciting a "particular cyclin" was considered unclear.

The rejected claims are canceled, and while the new claims are directed to similar subject matter, they do not include the language that provided the grounds for rejection under 35 U.S.C. §112, second paragraph. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, second paragraph, be withdrawn.

The rejections raised under 35 U.S.C. §112, second paragraph, have been addressed by the amendments and remarks above. Therefore, withdrawal of the rejections is respectfully requested.

Rejection of Claims Under 35 U.S.C. §102(b) as being anticipated by Bradley *et al.*:

Claim 18 was rejected under 35 U.S.C. §102(b) as being anticipated by Bradley *et al.* (1992), which discloses mouse embryonic stem (ES) cell lines that display germline transmission.

The examiner contends that the claimed cells do not possess any particular features or characteristics that distinguished them from the mouse ES cell lines disclosed by Bradley et al.

Although claim 18 is canceled, Applicants address the rejection as it could apply to new claims 79, 83, 112, 116, 117, 120, 121, 128, and 131, that are directed to embryonic cells of the present invention that encompass cells having murine genomic DNA. Applicants respectfully submit that the claimed cells are distinct from the cells disclosed by Bradley et al., because they are have the genomic DNA of one species of mammal and cytoplasm inherited from an oocyte of a different species of mammal. Such cells are useful for studying the differentiation of embryonic cells (as described on page 10, lines 1-3 of the specification) in ways that are not possible with cells having genomic DNA and cytoplasm of the same species, such as the murine ES cells disclosed by Bradley et al.

Differences between the developmental/differentiation potential of embryonic cells produced by cross-species nuclear transfer and embryonic cells having nuclei and cytoplasm of the same species are evident in the experimental results reported by Dominko et al. (Biology of Reproduction 60:1496-1502, 1999; and International Publication No. WO 99/05266, published Feb. 4, 1999, copies of which are attached). Dominko et al. transferred differentiated donor cells (fibroblasts) from cows, sheep, pigs, monkeys, and rats into enucleated bovine oocytes and studied the subsequent development of the resulting nuclear transfer (NT) units, in order to obtain better understanding of cell cycle compatibilities between genomes of donor cells and cytoplasm of recipient oocytes after nuclear transfer. They reported that blastocysts were generated from NT units produced by transfer of nuclei of cows, sheep, pigs, and monkeys into enucleated bovine oocytes, but not by transfer of rat nuclei into bovine oocytes (Table 1, page 1499). They observed that the timing of the first two mitotic divisions of the cross-species NT units appeared to be under control of the bovine

oocyte, but that timing of subsequent divisions and of the onset of the blastocoel cavity appeared to be under genomic control and reflected the timing of these events of the species of the nuclear donor cell (see p. 1498, right column). They also reported that blastocysts generated from the cross-species NT units failed to develop into fetuses after being transferred to surrogate female animals (see p. 1499, left column).

As discussed by Dominko et al., in order for the genome of a differentiated donor cell to direct development of an embryo formed by nuclear transfer into an enucleated oocyte, the donor chromatin must be "re-programmed" to an embryonic state by factors present in the oocyte cytoplasm. While the extent to which the factors that mediate such reprogramming operate in a species-specific manner is not understood, the results reported by Dominko et al. show that factors in bovine oocytes are capable of reprogramming the chromatin of differentiated cells of three other mammalian species - sheep, pigs, and monkeys, but not of rats, so that it is able to sustain embryogenesis leading to formation of a "blastocyst-like structure with distinct blastocyst morphology," including an inner cell mass, trophectoderm, and blastocoel cavity (Dominko et al., page 1500).

Early embryogenetic events occur in the absence of transcription of the embryonic genome, and are controlled by maternally inherited gene products in the oocyte cytoplasm. The time during embryogenesis at which transcription of embryonic genes begins is referred to as the "maternal to embryonic" (MET) transition, and occurs at a species-specific developmental stage. For example, transcription begins in bovine and porcine embryos at the late 4- to early 8-cell stage, in murine and rat embryos at the 2- to 4-cell stage, in sheep embryos at the 8- to 16-cell stage, and in human embryos at the 4- to 8-cell stage (see Dominko et al., page 1500, left column; and page 13 of International Publication No. WO 99/05266, published Feb. 4, 1999). The dependence of the timing of later embryogenetic events on the species of the nuclear donor cell observed by Dominko et al. further

demonstrates that the biochemical metabolism within the cells of the cross-species embryos is the result of species-specific interactions between the genomic DNA and oocyte-derived factors.

The complement of proteins that is initially produced by the embryonic genome also varies according to the species of the embryo, and the initial pattern of embryonic gene expression is thought to be regulated by species-specific factors present in the oocyte cytoplasm (Int. Pub. No. WO 99/05266, paragraph bridging pages 13-14). Species-specific incompatibilities between the oocyte-derived mitochondria and proteins expressed by nuclear genes are another possible reason for species-specific differences in the development and differentiation of embryonic cells of embryos produced by cross-species nuclear transfer. Mammalian mitochondrial DNA codes for 13 enzymes that mediate oxidative phosphorylation, 22 tRNAs, and two rRNAs (Smith et al., J. Reprod. Fertil. Suppl. 48:31-43, 1993, abstract attached). Kenyon et al. show that oxidative phosphorylation is impaired in cells with human genomic DNA and mitochondria of orangutan, New World monkeys, or lemurs, but not in cells with human genomic DNA and mitochondria of gorillas or chimpanzees (Proc. Nat. Acad. Sci. U.S.A., 94:9131-9135, 1997, see pages 9132-9133; copy attached). Dominko et al. state that before the usefulness of cross-species nuclear transfer (e.g., to generate cells and tissue for transplantation, etc.) can be evaluated, it is necessary to determine "the extent and faithfulness of nuclear reprogramming," and other compatibilities between the somatic cell's genetic information and factors in the recipient oocyte cytoplasm, including the mitochondria. Dominko et al. conclude with the statement that embryonic cell lines grown from cross-species embryos are expected to be useful for evaluating "long- and short-term effects of mixing of nuclear and cytoplasmic components of various species" (Dominko et al., page 1501).

As taught by Dominko et al., cells of embryos produced by cross-species nuclear transfer are metabolically distinct from normal embryonic cells, and studies of differentiation of cells isolated from embryos produced by cross-species nuclear transfer, such as those of the present invention, can provide useful information about the extent and faithfulness of chromatin reprogramming in cross-species embryos, and about inter-specific compatibilities between the complement of genome-encoded proteins and organelles and proteins of the ooplasm. Accordingly, Applicants submit that the claimed cells are not anticipated by normal ES cells, and respectfully request that the rejection of claims under 35 U.S.C. §102(b) as being anticipated by Bradley *et al.* be withdrawn.

Rejection of Claims Under 35 U.S.C. §102(e) as being anticipated by Tsukamoto *et al*:

Claims 18–25 were rejected under 35 U.S.C. §102(e) as being anticipated by Tsukamoto *et al.* (U.S. Pat. No. 5,716,827), which discloses human hematopoietic stem cells, on the ground that the phrase (human embryonic or stem-like cells" in the rejected claims could be construed to include human hematopoietic stem cells. Rejected claims 18–25 are canceled, and while the new claims encompass embryonic cells having human genomic DNA and non-human mitochondrial DNA, they do not include claims that broadly recite human stem-like cells that would include the human hematopoietic stem cells disclosed by Tsukamoto *et al.* Accordingly, Applicants respectfully request that the rejection of claims under 35 U.S.C. §102(e) as being anticipated by Tsukamoto *et al.* be withdrawn.

Rejection of Claims Under 35 U.S.C. §102(a) as being anticipated by Granerus et al.:

Claims 18–23 were rejected under 35 U.S.C. §102(a) as being anticipated by Granerus et al. (1996), which discloses a human teratoma cell line. As discussed above, the

metabolism of the claimed cells is distinct from that of 'normal" cells having genomic DNA and cytoplasm of the same species. The claimed cells can be produced and studied to provide useful information about chromatin reprogramming in cross-species embryos, and about inter-specific compatibilities between the set of proteins encoded by the genomic DNA, and the organelles and proteins of oocyte cytoplasm. Such studies are not possible with the teratoma cells disclosed by Granerus et al. Accordingly, Applicants submit that the claimed cells are not anticipated by human teratoma cells, and respectfully request that the rejection of claims under 35 U.S.C. §102(a) as being anticipated by Granerus et al. be withdrawn.

Rejection of Claims Under 35 U.S.C. §102(b) as being anticipated by Yamane:

Claims 18–25 were also rejected under 35 U.S.C. §102(b) as being anticipated by Yamane (1987), which discloses primary cultures of human differentiated cells (abstract on page 219).

Applicants submit that the cells isolated from embryos produced by cross-species nuclear transfer of the present invention are distinct from the normal, human differentiated cells disclosed by Yamane. As discussed above, unlike the cells disclosed by Yamane, the claimed cells can be studied to provide useful information about the extent and faithfulness of chromatin reprogramming in cross-species embryos, and about inter-specific compatibilities between the set of proteins encoded by the genomic DNA, and proteins and organelles of the oocyte cytoplasm. Accordingly, Applicants submit that the claimed cells are not anticipated by normal human cells, and respectfully request that the rejection of claims under 35 U.S.C. §102(b) as being anticipated by Yamane be withdrawn.

Rejection of Claim 31 Under 35 U.S.C. §103(a) as being unpatentable over Tsukamoto *et al.*:

Claim 31 was rejected under 35 U.S.C. 103(a) as being unpatentable over Tsukamoto *et al.* (U.S. Pat. No. 5,716,827). Claim 31, directed to differentiated, genetically altered human cells, is canceled, and the new claims do not recite such differentiated human cells. Tsukamoto *et al.* neither discloses nor suggests making or using the claimed cells produced by cross-species nuclear transfer. Accordingly, Applicants respectfully request that the rejection of claims under 35 U.S.C. §103(a) as being are not unpatentable over Tsukamoto *et al.* be withdrawn.

Rejection of Claims 1-25 Under 35 U.S.C. §103(a) as being unpatentable over Wolfe *et al.*, taken with Collas et al.:

Claims 1–25 were rejected under 35 U.S.C. 103(a) as being unpatentable over Wolfe *et al.* (1990) in view of Collas et al. (1994).

Although claims 1-25 are canceled, Applicants address the rejection as it could apply to the new claims.

Wolfe *et al.* show that cross-species nuclear transfer of embryonic nuclei may produce a nuclear transfer unit that can generate a blastocyst, and Collas et al. teach cross-species nuclear transfer using a differentiated donor cell. Prior to the present invention, no one had that a blastocyst could be produced cross-species nuclear transfer using a differentiated mammalian cell as the nuclear donor cell, and that lines of embryonic cells could be produced by culturing embryonic cells derived from such a blastocyst.

Those skilled in the art recognize that the chromatin of embryonic donor cells such as those used by Wolfe et al. is already in an embryonic configuration, and does not require

significant reprogramming by the oocyte cytoplasm to be capable of directing embryogenesis, whereas the chromosomes of differentiated donor cells are in a non-embryonic state, and must be reprogrammed. One of ordinary skill in the art would have known that in order for the DNA of a differentiated donor cell to successfully direct embryogenesis, the chromatin of the donor cell must be reprogrammed by the oocyte cytoplasm, the factors in the oocyte cytoplasm that activate expression of genomic genes required for embryogenesis must be compatible with the chromatin proteins and DNA sequences of the donor cell chromatin, the proteins encoded by the donor chromatin must be metabolically compatible with proteins and organelles such as mitochondria of the oocyte; and the reprogramming and activation of the donor cell chromatin must occur with timing that is conducive to successful blastogenesis.

Until Applicants successfully demonstrated the present invention, it was not possible for one of ordinary skill in the art topredict whether or not the claimed methods could be practiced with a reasonable expectation of success. Accordingly, the invention could not have been prima facie obvious. Therefore, Applicants respectfully request that the rejection of claims under 35 U.S.C. §103(a) be withdrawn.

All issues raised by the Office Action dated January 30, 2002, have been addressed in this Reply. Accordingly, a Notice of Allowance is next in order. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that she contact the undersigned so that such issues may be addressed expeditiously.

Respectfully submitted,
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